Claims:

1. A nucleic acid vector comprising:
first and second nucleotide sequences corresponding to nucleotide sequences flanking a predetermined insertion site in the RL1 locus of the genome of a selected herpes simplex virus (HSV); and a cassette located between said first and second nucleotide sequences comprising nucleic acid encoding:
   (a) one or a plurality of insertion sites; and
   (b) a ribosome binding site; and
   (c) a marker,
wherein the nucleic acid encoding the one or plurality of insertion sites is/are arranged upstream (5′) of the ribosome binding site and the nucleic acid encoding the ribosome binding site is arranged upstream (5′) of the marker.

2. A nucleic acid vector comprising:
first and second nucleotide sequences corresponding to nucleotide sequences flanking a predetermined insertion site in the RL1 locus of the genome of a selected herpes simplex virus (HSV); and a nucleic acid cassette located between said first and second nucleotide sequences comprising:
   (a) a third nucleotide sequence being of interest; and nucleic acid encoding:
   (b) a ribosome binding site; and
   (c) a marker,
wherein the nucleotide sequence of interest is arranged upstream (5′) of the ribosome binding site and the ribosome binding site is arranged upstream (5′) of the marker.

3. A vector as claimed in claim 1 or claim 2 wherein the ribosome binding site comprises an internal ribosome entry site (IRES).

4. A nucleic acid vector comprising:
first and second nucleotide sequences corresponding to nucleotide sequences flanking a predetermined insertion site in the RL1 locus of the genome of a selected herpes simplex virus (HSV); and a cassette located between said first and second nucleotide sequences comprising nucleic acid encoding:

(a) one or a plurality of insertion sites; and

(b) a regulatory nucleotide sequence; and

(c) a marker,

wherein the nucleic acid encoding the one or plurality of insertion sites is/are arranged upstream (5′) of the regulatory nucleotide sequence and the nucleic acid encoding the regulatory nucleotide sequence is arranged upstream (5′) of the marker.

5. A nucleic acid vector comprising:
first and second nucleotide sequences corresponding to nucleotide sequences flanking a predetermined insertion site in the RL1 locus of the genome of a selected herpes simplex virus (HSV); and a nucleic acid cassette located between said first and second nucleotide sequences comprising:

(a) a third nucleotide sequence being of interest; and nucleic acid encoding:

(b) a regulatory nucleotide sequence; and

(c) a marker,

wherein the nucleotide sequence of interest is arranged upstream (5′) of the regulatory nucleotide sequence and the regulatory nucleotide sequence is arranged upstream (5′) of the marker.

6. A vector as claimed in claim 4 or claim 5 wherein said regulatory nucleotide sequence is operably linked to said marker.

7. A vector as claimed in any one of claims 4 to 6 wherein said regulatory nucleotide sequence comprises a constitutive or inducible promoter.

8. A vector as claimed in claim 2 or claim 5 wherein the nucleotide sequence of interest encodes an heterologous polypeptide.

9. A vector as claimed in claim 8 wherein the heterologous polypeptide is selected from the group consisting of: a bacterial polypeptide; a mammalian polypeptide; a human polypeptide.

10. A vector as claimed in claim 8 wherein the heterologous polypeptide is selected from the group consisting of: Sodium iodide symporter (NIS); Nitroreductase (NTR); E.coli NTR; Endothelial nitric oxide synthase (eNOS); Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF); a cytokine.

11. A vector as claimed in claim 2 or claim 5 wherein the nucleotide sequence of interest encodes a selected antisense nucleic acid or siRNA.

12. A vector as claimed in any one of claims 2, 3, 5, 8 to 11 wherein the cassette further comprises a regulatory nucleotide sequence located upstream (5′) of the nucleotide sequence of interest which has a role in regulating transcription of the nucleotide sequence of interest.

13. A vector as claimed in any one of claims 1, 3, 4, 6 or 7 wherein the cassette further comprises a regulatory nucleotide sequence located upstream (5′) of the insertion site(s).

14. A vector as claimed in any one of claims 1, 3, 4, 6, 7, or 13 wherein the cassette comprises a plurality of said insertion sites.

15. A vector as claimed in any one of claims 1, 3, 4, 6, 7, 13 or 14 wherein each insertion site is formed by nucleic acid encoding a restriction endonuclease site.

16. A vector as claimed in any one of claims 1, 3, 4, 6, 7, 13 , 14 or 15 wherein the insertion sites comprise one or more of the ClaI, BglII, NruI and XhoI restriction endonuclease sites.

17. A vector as claimed in any one of claims 1 to 16 wherein the first and second nucleotide sequences each comprise sequence corresponding to nucleotide sequences in the RL terminal or internal repeat region of the genome of the selected HSV.

18. A vector as claimed in any one of claims 1 to 17 wherein said first and second nucleotide sequences

correspond to nucleotide sequences flanking a predetermined insertion site formed in, or comprising all or a part of, the ICP34.5 protein coding sequence of the genome of a selected herpes simplex virus.

19. A vector as claimed in any one of claims 1 to 18 wherein said first and second nucleotide sequences comprise contiguous portions of nucleotide sequence of the ICP34.5 gene of a herpes simplex virus.

20. A vector as claimed in any one of claims 1 to 19 wherein said first and second nucleotide sequences comprise contiguous portions of nucleotide sequence encoding the ICP34.5 gene product of a herpes simplex virus.

21. A vector as claimed in any one of claim 1 to 20 wherein the first and second nucleotide sequences have at least 60% sequence identity to their corresponding sequence in the viral genome.

22. A vector as claimed in any one of claims 1 to 20 wherein said first and second nucleotide sequences hybridise to their corresponding nucleotide sequence in the HSV genome, or its complement, under high or very high stringency conditions.

23. A vector as claimed in any one of claims 1 to 22 wherein the marker is a defined nucleotide sequence encoding a polypeptide.

24. A vector as claimed in any one of claims 1 to 23 wherein the marker comprises the Green Fluorescent

Protein (GFP) protein coding sequence or the enhanced Green Fluorescent Protein (EGFP) protein coding sequence.

25. A vector as claimed in any one of claims 1 to 22 wherein the marker comprises a defined nucleotide sequence detectable by hybridisation under high stringency conditions with a corresponding labelled nucleic acid probe.

26. A vector as claimed in any one of claims 1 to 25 wherein the cassette further comprises nucleic acid encoding a polyadenylation sequence located downstream (3′) of the nucleic acid encoding the marker.

27. A vector as claimed in claim 26 wherein the polyadenylation sequence comprises the Simian Virus 40 (SV40) polyadenylation sequence.

28. A vector as claimed in any one of the preceding claims wherein the vector further comprises nucleic acid encoding a second selectable marker.

29. A vector as claimed in any one of the preceding claims wherein the vector is a DNA vector, particularly a dsDNA vector.

30. Plasmid RL1.dIRES-GFP (ECACC accession number 03090303).

31. A vector as claimed in any one of the preceding claims wherein the vector is an expression vector.

32. A method of generating a herpes simplex virus which expresses a nucleotide sequence of interest, or polypeptide thereby encoded, comprising the step of culturing a selected herpes simplex virus with a vector as claimed in any one of claims 1 to 31, thereby integrating components (a), (b) and (c) of said vector at said predetermined insertion site in the genome of the selected herpes simplex virus.

33. The method of claim 32 wherein said herpes simplex virus is an HSV-1 or HSV-2.

34. The method of claim 32 or 33 wherein the integrated components disrupt a protein coding sequence resulting in inactivation or lack of expression of the respective gene product in the generated virus.

35. The method of any one of claims 32 to 34 wherein the generated herpes simplex virus is a gene specific null mutant.

36. The method of any one of claims 32 to 35 wherein the generated herpes simplex virus is an ICP34.5 null mutant.

37. The method of any one of claims 32 to 36 wherein the generated herpes simplex virus lacks at least one expressible ICP34.5 gene.

38. The method of any one of claims 32 to 34 wherein the generated herpes simplex virus lacks only one expressible ICP34.5 gene.

39. The method of any one of claims 32 to 37 wherein the generated herpes simplex virus is non-neurovirulent.

40. A vector as claimed in any one of claims 1 to 31 for use in a method of medical treatment.

41. A vector as claimed in any one of claims 1 to 31 for use in the treatment of cancer.

42. A vector as claimed in any one of claims 1 to 31 for use in the oncolytic treatment of a tumour.

43. A vector as claimed in any one of claims 1 to 31 for use in gene therapy.

44. Use of a vector as claimed in any one of claims 1 to 31 in the manufacture of a medicament for the treatment of disease.

45. Use of a vector as claimed in any one of claims 1 to 31 in the manufacture of a medicament for the treatment of cancer.

46. The use claimed in claim 45 wherein said medicament comprises a mutant herpes simplex virus generated using said vector.

47. A kit of parts comprising a first container having a quantity of a vector as claimed in any one of claims 1 to 31 and a second container comprising a quantity of herpes simplex virus genomic DNA.

48. An herpes simplex virus (HSV) wherein the herpes simplex virus comprises a nucleic acid cassette integrated in the RL1 locus of the HSV genome comprising nucleic acid encoding:

(a) one or a plurality of insertion sites; and

(b) a ribosome binding site, and a

(c) marker,

wherein the nucleic acid encoding the one or plurality of insertion sites is/are arranged upstream (5′) of the ribosome binding site and the nucleic acid encoding the ribosome binding site is arranged upstream (5′) of the marker.

49. An herpes simplex virus (HSV) wherein the herpes simplex virus comprises a nucleic acid cassette integrated in the RL1 locus of the HSV genome comprising nucleic acid encoding:

(a) a nucleotide sequence of interest; and nucleic acid encoding:

(b) a ribosome binding site; and

(c) a marker,

wherein the nucleotide sequence of interest is arranged upstream (5′) of the ribosome binding site and the ribosome binding site is arranged upstream (5′) of the marker.

50. A vector as claimed in claim 48 or claim 49 wherein the ribosome binding site comprises an internal ribosome entry site (IRES).

51. An herpes simplex virus as claimed in any one of claims 49 or 50 wherein a transcription product of the cassette is a bi- or poly- cistronic transcript

comprising a first cistron encoded by the nucleotide sequence of interest and a second cistron encoded by the marker nucleic acid wherein the ribosome binding site is located between said first and second cistrons.

52. An herpes simplex virus (HSV) wherein the herpes simplex virus comprises a nucleic acid cassette integrated in the RL1 locus of the HSV genome comprising nucleic acid encoding:

(a) one or a plurality of insertion sites; and

(b) a regulatory nucleotide sequence; and

(c) a marker,

wherein the nucleic acid encoding the one or plurality of insertion sites is/are arranged upstream (5′) of the regulatory nucleotide sequence and the nucleic acid encoding the regulatory nucleotide sequence is arranged upstream (5′) of the marker.

53. An herpes simplex virus (HSV) wherein the herpes simplex virus comprises a nucleic acid cassette integrated in the RL1 locus of the HSV genome comprising nucleic acid encoding:

(a) a nucleotide sequence of interest; and nucleic acid encoding:

(b) a regulatory nucleotide sequence; and

(c) a marker,

wherein the nucleotide sequence of interest is arranged upstream (5′) of the regulatory nucleotide sequence and the regulatory nucleotide sequence is arranged upstream (5′) of the marker.

54. An herpes simplex virus as claimed in claim 52 or claim 53 wherein said regulatory nucleotide sequence is operably linked to said marker.

55. An herpes simplex virus as claimed in any one of claims 52 to 54 wherein said regulatory nucleotide sequence comprises a constitutive or inducible promoter.

56. An herpes simplex virus as claimed in any one of claims 49 to 51 or 53 to 55 wherein the nucleotide sequence of interest encodes an heterologous polypeptide.

57. An herpes simplex virus as claimed in claim 56 wherein the heterologous polypeptide is selected from the group consisting of: a bacterial polypeptide; a mammalian polypeptide; a human polypeptide.

58. An herpes simplex virus as claimed in claim 56 wherein the heterologous polypeptide is selected from the group consisting of: Sodium iodide symporter (NIS); Nitroreductase (NTR); E.coli NTR; Endothelial nitric oxide synthase (eNOS); Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF); a cytokine.

59. An herpes simplex virus as claimed in any one of claims 49 to 51 or 53 to 55 wherein the nucleotide sequence of interest encodes a selected antisense nucleic acid or siRNA.

60. An herpes simplex virus as claimed in any one of claims 49, 50, 51, 53, 56 to 59 wherein the cassette further comprises a regulatory nucleotide sequence located upstream (5′) of the nucleotide sequence of

interest which has a role in regulating transcription of the nucleotide sequence of interest.

61. An herpes simplex virus as claimed in any one of claims 48, 50, 51, 52, 54 or 55 wherein the cassette further comprises a regulatory nucleotide sequence located upstream (5′) of the insertion site(s).

62. An herpes simplex virus as claimed in any one of claims 48, 50, 51, 52, 54, 55 or 61 wherein the cassette comprises a plurality of said insertion sites.

63. An herpes simplex virus as claimed in any one of claims 48, 50, 51, 52, 54, 55, 61 or 62 wherein each insertion site is formed by nucleic acid encoding a restriction endonuclease site.

64. An herpes simplex virus as claimed in any one of claims 48, 50, 51, 52, 54, 55, 61, 62 or 63 wherein the insertion sites comprise one or more of the ClaI, BglII, NruI and XhoI restriction endonuclease sites.

65. An herpes simplex virus as claimed in any one of claims 48 to 64 wherein the nucleic acid cassette is integrated in the RL terminal or internal repeat region of the genome of the selected HSV.

66. An herpes simplex virus as claimed in any one of claims 48 to 65 wherein the nucleic acid cassette is integrated at a site formed in, or comprising all or a part of, the ICP34.5 protein coding sequence of the genome of a selected herpes simplex virus.

67. An herpes simplex virus as claimed in any one of claims 48 to 66 wherein the nucleic acid cassette is integrated in the genomic nucleotide sequence of the ICP34.5 gene of a herpes simplex virus.

68. An herpes simplex virus as claimed in any one of claims 48 to 67 wherein the nucleic acid cassette is integrated in the genomic nucleotide sequence encoding the ICP34.5 gene product of a herpes simplex virus.

69. An herpes simplex virus as claimed in any one of claims 48 to 68 wherein the marker is a defined nucleotide sequence encoding a polypeptide.

70. An herpes simplex virus as claimed in any one of claims 48 to 69 wherein the marker comprises the Green Fluorescent Protein (GFP) protein coding sequence or the enhanced Green Fluorescent Protein (EGFP) protein coding sequence.

71. An herpes simplex virus as claimed in any one of claims 48 to 68 wherein the marker comprises a defined nucleotide sequence detectable by hybridisation under high stringency conditions with a corresponding labelled nucleic acid probe.

72. An herpes simplex virus as claimed in any one of claims 48 to 71 wherein the cassette further comprises nucleic acid encoding a polyadenylation sequence located downstream (3′) of the nucleic acid encoding the marker.

73. An herpes simplex virus as claimed in claim 72 wherein the polyadenylation sequence comprises the Simian Virus 40 (SV40) polyadenylation sequence.

74. An herpes simplex virus as claimed in any one of claims 48 to 73 wherein the cassette disrupts a protein coding sequence in the HSV genome resulting in inactivation of the respective gene product.

75. An herpes simplex virus as claimed in any one of claims 48 to 74 wherein the herpes simplex virus is a mutant of HSV-1 or HSV-2.

76. An herpes simplex virus as claimed in any one of claims 48 to 75 wherein the herpes simplex virus is a mutant of one of HSV-1 strains 17 or F or HSV-2 strain HG52.

77. An herpes simplex virus as claimed in any one of claims 48 to 76 which is a gene specific null mutant.

78. An herpes simplex virus as claimed in any one of claims 48 to 77 which is an ICP34.5 null mutant.

79. An herpes simplex virus as claimed in any one of claims 48 to 78 which lacks at least one expressible ICP34.5 gene.

80. An herpes simplex virus as claimed in any one of claims 48 to 76 which lacks only one expressible ICP34.5 gene.

81. An herpes simplex virus as claimed in any one of claims 48 to 79 which is non-neurovirulent

82. An herpes simplex virus as claimed in any one of claims 48 to 81 for use in a method of medical treatment.

83. An herpes simplex virus as claimed in any one of claims 48 to 81 for use in the treatment of cancer.

84. An herpes simplex virus as claimed in any one of claims 48 to 81 for use in the oncolytic treatment of a tumour.

85. Use of an herpes simplex virus as claimed in any one of claims 48 to 81 in the manufacture of a medicament for the treatment of cancer.

86. A method of lysing or killing tumour cells in vitro or in vivo comprising the step of administering to a patient in need of treatment a therapeutically effective amount of an herpes simplex virus as claimed in any one of claims 48 to 81.

87. A medicament, pharmaceutical composition or vaccine comprising an herpes simplex virus as claimed in any one of claims 48 to 81.

88. A medicament, pharmaceutical composition or vaccine as claimed in claim 87 further comprising a pharmaceutically acceptable carrier, adjuvant or diluent.

89. A method of generating a nucleic acid vector comprising the steps of:

i) providing a first nucleotide sequence comprising a predetermined second nucleotide sequence corresponding to a selected nucleotide sequence in the RL1 locus of the genome of a selected Herpes simplex virus; and

ii) inserting nucleotide sequence(s) in said second nucleotide sequence encoding:

a) one or a plurality of insertion sites and/or a nucleotide sequence of interest; and

b) a ribosome binding site or a regulatory nucleotide sequence; and

c) a marker,

wherein the insertion site(s)/nucleotide sequence of interest is arranged upstream (5′) of the ribosome binding site/ regulatory nucleotide sequence and the ribosome binding site / regulatory nucleotide sequence is arranged upstream (5′) of the marker.

90. The method of claim 89 wherein the inserted nucleotide sequence(s) separates the second nucleotide sequence into two vector flanking sequences, the inserted nucleotide sequences forming a cassette therebetween.

91. The method as claimed in claim 89 or claim 90 wherein the second nucleotide sequence corresponds to a nucleotide sequence in the RL terminal or internal repeat region of the genome of the selected herpes simplex virus.

92. The method as claimed in any one of claims 89 to 91 wherein the second nucleotide sequence corresponds to all or a part of the ICP34.5 protein coding sequence of the genome of the selected herpes simplex virus.

93. The method as claimed in any one of claims 89 to 92 wherein said second nucleotide sequence comprises a contiguous portion of nucleotide sequence of the ICP34.5 gene of the selected herpes simplex virus.

94. The method as claimed in any one of claims 91 to 93 wherein said second nucleotide sequence comprises a contiguous portion of nucleotide sequence encoding the ICP34.5 gene product of the selected herpes simplex virus.

95. The method as claimed in any one of claim 89 to 94 wherein the second nucleotide sequence has at least 60% sequence identity to the corresponding sequence in the viral genome.

96. The method as claimed in any one of claims 89 to 94 wherein said second nucleotide sequence hybridises to the corresponding nucleotide sequence in the viral genome, or its complement, under high or very high stringency conditions

97. A method of generating a mutant herpes simplex virus (HSV) comprising inserting a nucleic acid cassette comprising nucleotide sequence(s) encoding:

a) one or a plurality of insertion sites and/or a nucleotide sequence of interest; and

b) a ribosome binding site or a regulatory nucleotide sequence; and

c) a marker

into a predetermined insertion site in the RL1 locus of the genome of a selected HSV, wherein the insertion

site(s)/nucleotide sequence of interest is arranged upstream (5′) of the ribosome binding site/ regulatory nucleotide sequence and the ribosome binding site/ regulatory nucleotide sequence is arranged upstream (5′) of the marker.

98. The method of claim 97 wherein said method comprises the steps of:

i) providing a vector as claimed in any one of claims 1 to 31;

ii) where the vector is a plasmid, linearising the vector; and

iii) co-transfecting a cell culture with the linearised vector and genomic DNA from said selected HSV.

99. The method of claim 98 wherein said co-transfection is carried out under conditions effective for homologous recombination of said cassette into an insertion site in the viral genome.

100. The method of any one of claims 97 to 99 wherein said method further comprises one or more of the steps of:

1) screening said co-transfected cell culture to detect mutant HSV expressing said marker; and/or

2) isolating said mutant HSV; and/or

3) screening said mutant HSV for expression of the nucleotide sequence of interest or the RNA or polypeptide thereby encoded; and/or

4) screening said mutant HSV for lack of an active gene product; and/or

5) testing the oncolytic ability of said mutant HSV to kill tumour cells in vitro.

101. A method as claimed in any one of claims 97 to 100 wherein the nucleotide sequence of interest is heterologous to the selected herpes simplex virus.

102. The method as claimed in any one of claims 97 to 100 wherein the nucleotide sequence of interest encodes an heterologous polypeptide.

103. The method as claimed in claim 102 wherein the heterologous polypeptide is selected from the group consisting of: a bacterial polypeptide; a mammalian polypeptide; a human polypeptide.

104. The method as claimed in claim 102 wherein the heterologous polypeptide is selected from the group consisting of: Sodium iodide symporter (NIS); Nitroreductase (NTR); E.coli NTR; Endothelial nitric oxide synthase (eNOS); Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF); a cytokine.

105. The method as claimed in claim 101 wherein the nucleotide sequence of interest encodes a selected antisense nucleic acid or siRNA.

106. An herpes simplex virus generated by the method of any one of claims 97 to 105.

107. An herpes simplex virus gene specific null mutant generated by the method of any one of claims 97 to 105.

108. An herpes simplex virus ICP34.5 null mutant generated by the method of any one of claims 97 to 105.